On page 64, please replace paragraph 2 with the following:

The immunized mice were then treated with oligonucleotides (30 μg in 200 μl saline by i.p. injection), which either contained an unmethylated CpG motif (*i.e.*, TCCATGACGTTCCTGACGTT; SEQ ID NO.10) or did not (*i.e.*, control, TCCATGAGCTTCCTGAGTCT; SEQ ID NO. 8). Soluble SeEA (10 μg in 25 μl of saline) was administered by intranasal instillation on days 14 and 21. Saline was used as a control.

REMARKS

Claims 88-101 and 103-104 are pending. Claims 1, 42-88, 102, and 105-131 have been withdrawn from further consideration as being drawn to non-elected inventions. The specification has been amended to correct some minor errors recently identified. No new matter has been added.

SEQUENCE LISTING

Applicants have submitted a substitute sequence listing in computer readable diskette. It came to Applicants attention that several errors in the existing sequence listing needed to be corrected.

On page 1, a typographical error in the title was corrected.

On page 1, our reference number was updated.

On page 1, the number of sequences was updated due to an added sequence.

On page 10, SEQ ID NO: 41 was corrected due to a typographical error. The sequence was corrected to be consistent with the sequence shown in the specification in Table 9, page 36.

On page 22-23, SEQ ID NO: 107 (incorrectly labelled as SEQ ID NO: 117 in the specification in Table 1, page 22), was corrected due to a transposition error of the intended sequence.

On page 26, the sequence for oligonucletoide 5d (improperly labelled as SEQ ID NO: 114 in the specification in Table 3, page 26) was added as SEQ ID NO: 124.

FIGURES

The Examiner has indicated that Figs. 4B, 5, 6, 7, 8A, 8B, and 9-15 are missing from the specification. Applicant hereby encloses copies of Figs. 4B, 5, 6, 7, 8A, 8B, and 9-15 as

originally filed in U.S. Serial No. 08/960,774, filed on October 30, 1997, the entire contents of which was incorporated by reference. No new matter is added.

SPECIFICATION

Applicants have also amended the specification to update the related application information. Applicants have also amended several sections of the specification to correct minor errors.

On page 11, the description needed to be corrected to match the data and the sequence listing. The control oligonucleotide used in this section actually lacked a CpG motif rather than having a GpC motif.

On page 12, the control oligonucleotide was incorrectly listed as SEQ. ID No. 11. The specification has been amended to correct this error.

On page 22, and 26 the wrong SEQ. ID Nos. were listed. The specification has been amended to correct these errors.

On page 26 in Table 3, a period was missing in the sequence of oligonucleotide 5d and the sequence was incorrectly identified at 114 rather than 124..

On page 37, line 8 improperly recited 10-17 residues. This has been changed to residues 11-17. The error is clear from the sequence listed in the specification.

On page 64, the word "the" was replaced with the word -- not-- . The error was typographical in nature. The changes do not add any new matter.

Rejection of Claim 104 under 35 U.S.C.§ 112, 2nd ¶

Claim 104 has been rejected under 35 U.S.C. § 112, 2nd ¶ as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. According to the Examiner, the term "stabilized" is unclear. The term stabilized nucleic acid molecule is defined on pages 18-19 of the specification. The stabilized nucleic acid molecule is one which is relatively resistant to *in vivo* degradation. The preferred stabilized nucleic acid molecules of the invention has a modified backbone to reduce the rate of degradation. The meaning of the term stabilized is clear to those of skill in the art based on the teachings in the specification.

Rejection of Claims 88-101, 103, and 104 under 35 U.S.C. § 112, 1st ¶

Claims 88-101, 103, and 104 have been rejected under 35 U.S.C. § 112, 1st ¶ "because the specification, while being enabling for stimulating various immune associated responses, comprising B cell and monocyte activation, and producing the conversion from a Th2 to a Th1 type immune response in an organism, comprising the administration of an unmethylated CpG containing oligonucleotide, does not reasonably provide enablement for the treatment and prevention of any and/or all bacterial infections in an organism".

Claims 88-101 and 103-104 relate to methods for treating and preventing bacterial infection in a subject. The specification on page 9, lines 5-7 teaches that the CpG oligonucleotides of the invention are useful for treating and preventing bacterial infection. An extensive list of infectious bacteria is presented on pages 14-15. A detailed description of CpG immunostimulatory nucleic acids useful in treating immune deficiencies for the treatment and prevention of bacterial infection is presented on pages 15-17. Methods for determining the stimulation index of a particular CpG DNA as presented on pages 17-18 stabilize nucleic acids are described on page 18-19. Pages 20-42 describe actual working examples demonstrating B cell activation, NK activation, induction of cytokines, such as IL6, and IL12 using many different oligonucleotides containing CpG having different backbones, under different conditions, using different dosages. Although working examples are not required in a patent application, many working examples have been provided.

One of skill in the art would have no reason to doubt a correlation between the data presented in the specification and the claimed invention of treating or preventing bacterial infections. Applicant has clearly demonstrated that a variety of CpG containing oligonucleotides can stimulate B cell activation, NK cell activation, and cytokine induction. The attached paper (Exhibit 4) is a review article describing the effects of CpG in activating innate immune defenses against infections. (Krieg, A., *Annu. Rev. Immunol.*, 20:709 (2002)). The review article describes several studies in which CpG was used successfully to treat and prevent different types of infectious disease in vivo (p. 728-29). CpG functions by activating an immune response to attack and kill the invading bacteria. This general immune response should be effective against bacteria in general, rather than being limited to a specific type of bacteria.

The specification provides adequate guidance concerning route of administration, types of oligonucleotides and dosages. For example, Applicants have described the type of administration, e.g., see page 54, lines 6-20. Applicants have described times or frequencies of

administration, for example, see page 53, lines 5-11 and 19-25. Effective amounts and manner of determining effective amounts to obtain the desired effects are described in the specification, for example, page 54, line 21-page 55, line 1. Additionally, the *in vitro* assays described in the specification can easily be translated into *in vivo* dosing parameters for oligonucleotides by those of ordinary skill in the art. The correlation between *in vitro* assays for antisense oligonucleotides to that of *in vivo* dosing parameters for oligonucleotides was known in the art at the time the application was filed. The following research articles published between 1991 and 1994 describe *in vivo* administration of antisense oligonucleotides.

Bayever et al., *Antisense Research and Development*, 3:383-390 (1993), attached as Exhibit 1, and submitted to the patent office in an IDS, describes the systemic administration of an antisense oligonucleotide during a Phase I trial. The pharmacokinetics and clinical effects of the oligonucleotides were described in detail. The discussion concludes that "the recovery of up to approximately 60% of OL(1)p53 in urine during the 10-day infusion is consistent with the observations from preclinical studies in the rat (our unpublished observations, 1993) and monkey (Cornish et al., 1993)". (Page 388, second full paragraph in discussion.)

Cossum et al., *The Journal of Pharmacology and Experimental Therapeutics*, 269(1):89-94, attached as Exhibit 2, describes the pharmacokinetics of an antisense oligonucleotide after intradermal administration to rats. It is taught in the abstract that the "rate and characteristics of metabolism in the skin were similar to those observed in other tissues". The reference describes the tissue distribution and pharmacokinetics, metabolism and elimination of the oligonucleotide.

The following review article was published in 1995 but described many studies performed prior to 1995.

Agrawal et al., *Clinical Pharmacokinetics*, 28(1):7-16 1995, attached as Exhibit 5, is a review article describing *in vivo* administration and pharmacokinetics of oligonucleotides in monkeys, mice, rats and humans.

In view of the teachings found in the specification as well as the state of the art at the time of the invention concerning the administration of antisense oligonucleotides for therapeutic purposes, one of ordinary skill in the art would have been enabled to practice the full scope of the claims.

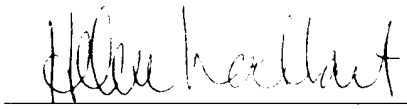
Double Patenting

Claim 104 has been rejected under the judicially created doctrine of obviousness-type double patenting. If claim 104 is found to be otherwise patentable, Applicants will consider filing a terminal disclaimer to overcome the rejection.

Summary

It is believed that all of the pending claims are now allowable. If the Examiner has any questions or comments, she is encouraged to contact Applicants' representative at the number listed below.

Respectfully submitted,



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Docket No. C01039.70042.US
Dated: September 30, 2002
x09/30/2002

MARKED-UP SPECIFICATION

On page 1, lines 3-9, please replace the heading "related application" and the paragraph following the heading with the following:

--Related applications

This application is a Divisional of U.S. Serial No. 08/960,774, filed October 30, 1997, which is now issued as U.S. Patent No. 6,239,116 B1 on May 29, 2001, which is a continuation-in-part of U.S. Serial No. 08/738,652, filed October 30, 1996, [pending], which is now issued as U.S. Patent No. 6,207,646 B1 on March 27, 2001, which is a continuation-in-part of U.S. Patent Application Serial No. 08/386,063, filed February 7, 1995 [currently pending], which is now issued as U.S. Patent No. 6,194,388 B1 on February 27, 2001, which is a continuation-in-part of U.S. Patent Application 08/276,358, filed July 15, 1994, which is now abandoned, each of which are incorporated herein by reference in their entirety.--

On page 11, please replace paragraph 3 with the following:

Figure 1A shows the results from a flow cytometry study using mouse B cells with the dihydrorhodamine 123 dye to determine levels of reactive oxygen species. The dye only sample in Panel A of the figure shows the background level of cells positive for the dye at 28.6%. This level of reactive oxygen species was greatly increased to 80% in the cells treated for 20 minutes with PMA and ionomycin, a positive control (Panel B). The cells treated with the CpG oligo (TCCATGACGTTCCTGACGTT SEQ ID NO: 10) also showed an increase in the level of reactive oxygen species such that more than 50% of the cells became positive (Panel D). However, cells treated with an oligonucleotide [with the identical sequence except that the CpGs were switched] that lacked a CpG motif (TCCATGAGCTTCCTGAGTCT SEQ ID NO: 8) did not show this significant increase in the level of reactive oxygen species (Panel E).

On page 12, please replace paragraph 2 with the following:

Figure 11 is a bar graph plotting the effect on the percentage of macrophage, lymphocyte, neutrophil and eosinophil cells induced by exposure to saline alone; egg, then SEA; egg and [SEQ ID NO: 11] SEQ ID NO: 10, then SEA; and egg and control oligo [(SEQ ID NO: 11)] SEQ ID NO: 8, then SEA. When the mice are treated with the control oligo at the time of the initial exposure to the egg, there is little effect on the subsequent influx of eosinophils into the lungs after inhalation of SEA. Thus, when mice inhale the eggs on days 14 or 21, they develop

an acute inflammatory response in the lungs. However, giving a CPG oligo along with the eggs at the time of initial antigen exposure on days 0 and 7 almost completely abolishes the increase in eosinophils when the mice inhale the egg antigen on day 14.

On page 22, please replace paragraph 5 of Table 1 with the following:

4	(SEQ ID NO:90)	TCAACGTT	6.1 ± 1.4	19.2 ± 5.2	
4a	(SEQ ID NO:91)		....GC..	1.1 ± 0.2	1.5 ± 1.1
4b	(SEQ ID NO:92)		...GCGC.	4.5.± 0.2	9.6 ± 3.4
4c	(SEQ ID NO:93)		...TCGA.	2.7.± 1.0	ND
4d	(SEQ ID NO:94)		..TT..AA	1.3 ± 0.2	ND
4e	(Residue 2-8 of SEQ ID NO:90; SEQ ID NO: 106)		-.....	1.3 ± 0.2	1.1 ± 0.5
4f	(SEQ ID NO:95)		C.....	3.9 ± 1.4	ND
4g	(Residue 11-18 of SEQ ID NO:19; SEQ ID NO:[117]) 107		--.....CT	1.4 ± 0.3	ND
4h	(SEQ ID NO:96)		C	1.2 ± 0.2	ND

On page 26, please replace Table 3 with the following:

Table 3. Induction of Murine IL-6 secretion by CpG motifs in bacterial DNA or oligonucleotides.

Treatment	IL-6 (pg/ml)
calf thymus DNA	≤10
calf thymus DNA + DNase	≤10
E. coli DNA	1169.5 ± 94.1
E. coli DNA + DNase	≤10
CpG methylated *E. coli* DNA	≤10
LPS	280.1 ± 17.1
Media (no DNA)	≤10
5a SEQ. ID. No:115	ATGGACTCTCCAGCGTTCTC 1096.4 ± 372.0
5b SEQ. ID. No:19	.....AGG....A..... 1124.5 ± 126.2
5c SEQ. ID. No:15	..C.....G..... 1783.0 ± 189.5
5d SEQ. ID. No:[114] 124	.AGG..C..T..... ≤10
5e SEQ. ID. No:116	..C.....G..Z..... 851.1 ± 114.4

5f SEQ. ID. No:16	..Z.....ZG..Z.....	≤10
5g SEQ. ID. No:18	..C.....G.....Z..	1862.3 + 87.26

On page 37, please replace paragraph 2 with the following:

Immune activation by CpG motifs may depend on bases flanking the CpG, and the number of spacing of the CpGs present within an ODN. Although a single CpG in an ideal base context can be a very strong and useful immune activator, superior effects can be seen with ODN containing several CpGs with the appropriate spacing and flanking bases. For activation of murine B cells, the optimal CpG motif is TGACGTT (SEQ. ID. NO: 108); residues [10-17] 11-17 of Seq. ID. No 70.

On page 64, please replace paragraph 2 with the following:

The immunized mice were then treated with oligonucleotides (30 μg in 200 μl saline by i.p. injection), which either contained an unmethylated CpG motif (*i.e.*, TCCATGACGTTCCTGACGTT; SEQ ID NO.10) [or did the] or did not (*i.e.*, control, TCCATGAGCTTCCTGAGTCT; SEQ ID NO. 8). Soluble SeEA (10 μg in 25 μl of saline) was administered by intranasal instillation on days 14 and 21. Saline was used as a control.